EVALUATION OF CELL CULTURE EXERCISE PROTOCOLS USING A MECHANICAL STRETCH SYSTEM FOR ANALYSIS OF DEPTOR EXPRESSION

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ABSTRACT

Evaluation of Cell Culture Exercise Protocols using a Mechanical Stretch System for Analysis of DEPTOR Expression. (May 2013)

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The generation and maintenance of muscle mass is a subject of interest across many scientific fields, including cancer, diabetes, and exercise physiology. Protein synthesis is a key step in the development and maintenance of muscle tissue, and alterations that affect this process can induce a wide array of significant and often deleterious results. A wide variety of regulatory proteins are involved in muscle tissue formation and protein synthesis. One such regulatory protein of particular interest is DEPTOR, which acts as a negative inhibitor of mTOR expression and decreases the rate of protein synthesis. Recent data in our lab has revealed that DEPTOR is differentially expressed in scenarios of diabetes, disuse, and exercise, suggesting that DEPTOR may have a significant physiological role as a key regulator of protein synthesis. In this review, an analysis of numerous exercise protocols for achieving various physiological outcomes is provided, specifically targeting protocols whose results may provide an ideal basis for evaluating changes in DEPTOR expression in the provided scenarios. Following this evaluation, an

analysis of different inhibitory compounds and substrates will be provided to give a basis for additional means of altering DEPTOR expression, which can be incorporated into exercise protocols to study the combined effects of exercise and molecular interactions on DEPTOR signaling. The information provided in this review is intended to provide an understanding of various aspects of mechanically simulated exercise that can be used as parameters to study DEPTOR expression in muscle *in vitro*.

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NOMENCLATURE

mTOR	Mammalian Target of Rapamyacin
mTORC1	Mammalian Target of Rapamyacin Complex 1
mTORC2	Mammalian Target of Rapamyacin Complex 2
DEPTOR	DEP-Domain-Containing mTOR-Interacting Protein
mRNA	Messenger Ribonucleic Acid
tRNA	Transfer Ribonucleic Acid
eIF4E	Eukaryotic Initiation Factor 4E
4E-BP1	Eukaryotic Initiation Factor 4E (eIF4E)- Binding Protein 1
S6K1 (also p70S6K)	p70 Ribosomal S6 Kinase 1
ERK1/2	Extracellular Signal-Related Kinases 1&2
AMPK	5' Adenosine Monophosphate-Activated Protein Kinase
eEFK2	Eukaryotic Elongation Factor-2 Kinase
$SCF^{\beta\text{-}TrCP}$	Skp1-Cul1-F-Box Protein Beta-Transducin Repeat Containing Protein
PI3K	Phosphatidylinositide 3-Kinase
MEK	Mitogen-Activated Protein Kinase Kinase
KD	Knockdown
MEF	Mouse Embryonic Fibroblasts
HEK293	Human Embryonic Kidney 293 Cells

MPC	Muscle Progenitor Cells
ECM	Extracellular Matrix
Hz	Hertz

CHAPTER I

INTRODUCTION

The generation and maintenance of muscle mass is a subject of interest across many scientific fields, including cancer, diabetes, and exercise physiology. Protein synthesis is a key step in the development and maintenance of muscle tissue, and alterations that affect this process can induce a wide array of significant and often deleterious results. A wide variety of regulatory proteins are involved in muscle tissue formation and protein synthesis. One such regulatory protein of particular interest is DEPTOR, a negative inhibitor of mTOR expression that is known to reduce rates of protein synthesis. A growing body of data has highlighted DEPTOR's importance in cancer growth (5, 11, 16, 19). Recent data from our lab has revealed that DEPTOR is differentially expressed in scenarios of diabetes, disuse, and exercise *in vivo*, suggesting that DEPTOR may have a significant physiological role as a key regulator of protein synthesis. In this review, we provide an analysis of numerous approaches to exercise protocols for achieving various physiological outcomes, specifically targeting protocols that may provide optimal conditions for evaluating changes in DEPTOR expression. Following this evaluation, an analysis of different inhibitory compounds and substrates will be provided to give a basis for additional means of altering DEPTOR expression that can be incorporated into exercise protocols. With this information, we hope to provide a thorough understanding of the advantages and applications of various cell culture exercise models as well as their

potential contributions to the understanding of DEPTOR regulation and protein synthesis in an effectively replicable environment.

Cell culture

Numerous models exist for analysis of muscle development, growth, and function. Rodent, human, and cell culture models have all been well documented for analysis of muscle signaling and response, with each of them containing their own advantages and setbacks. Over the years, cell culture has evolved as a highly suitable model for analyzing basic mechanistic processes due to its ease of maintenance, quick turnover, and potential for manipulation. Numerous cell lines with different properties have been developed from a staggering variety of tissues and species, including muscle cells. These isolated and immortalized muscle cells *in vitro* can be grown and manipulated by the addition of external mechanical forces to imitate muscle contractions *in vivo*, thus enabling the design and execution of exercise studies with a much greater degree of ease than one would find in parallel rodent or human experiments. A wide variety of exercise regimes have been documented using mechanical and electrical forces to simulate muscle contractions in both 2D and 3D constructs (18).

Myogenesis

Like muscle growth *in vivo,* muscle grown in culture uses the process of myogenesis to generate mature myotubes, which can be exposed to a wide variety of external stimuli. The process of skeletal muscle development involves the formation of muscle fibers

from the fusion of committed myoblasts into formations of multinucleated fibers called myotubes. Myoblasts originate from primitive myotome cells produced by structures called somites during early embryonic development. The process of myogenesis begins when myoblasts exit the cell cycle and undergo differentiation, essentially establishing their fates as muscle cells regardless of differing cellular signals. Once differentiated, myoblasts lose the ability to divide for the remainder of their cell life; thus muscle regeneration is known only to arise from the activation of muscle stem cells, also known as satellite cells, in most organisms (1, 3). As myoblasts continue to develop, they align linearly and begin to fuse into multinucleated structures known as primary myotubes. While primary myotubes mature, secondary myotubes emerge from myoblasts remaining beneath the basal lamina of the primary myotubes. Throughout development, both primary and secondary myotubes continue to grow until they are approximately the same size to create mature myofibers. Satellite cells remain quiescent within the basal lamina of adult myofibers and are capable of reinitiating myogenesis by differentiating into new myoblasts. While myogenesis has been observed in mammalian embryonic development and to some degree in muscle wound recovery, extension of this process to muscle recovery in other medical research could hold significant potential (12).

Protein synthesis

Protein synthesis is a highly regulated molecular process that leads to the growth of muscle cells and tissue. Muscle tissue constantly undergoes both anabolism and catabolism, thus levels of net levels of protein synthesis must exceed levels of

degradation levels in order for growth to occur (24). Because the mechanisms of protein degradation are numerous and not fully understood, much research has focused on protein synthesis rates as a measurement of muscle growth. In brief, protein synthesis results from the transcription of nuclear DNA into messenger-RNA (mRNA), which is exported from the nucleus. The mRNA codes for specific amino acid sequences that are integrated into a two-part ribosomal complex, and these amino acids may be translated into new proteins with the assistance of transfer-RNA (tRNA). Upon completion of translation, numerous modifications including folding and chemical labeling convert polypeptide chains into completed functional proteins. Since proteins are required for cell growth and proliferation, as cells must pass a certain size threshold before they can begin division, rates of protein synthesis can act as temporal regulators of cell growth and division (6).

mTOR regulation

Due to the high energy requirements of protein synthesis and its products, the processes governing protein synthesis and cell proliferation have evolved to be highly sensitive to signals from both extracellular and intracellular factors such as environment, nutrition, stress, among several. One key regulatory factor involved in this process is the mammalian target of rapamycin (mTOR) complex, which stimulates protein synthesis and consequent cell growth (9). mTOR is known to be a central regulator of several pathways related to protein metabolism, survival, and cell growth, and recent studies over the past few years have highlighted the impact of its deregulation in diseases such

cancer and diabetes (7, 11). While mTOR is present in two different complexes, mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2), numerous studies have identified mTORC1 as the key regulator of protein synthesis and muscle mass determination. The mTOR complex incorporates signals from growth factors, energy status, and amino acid availability into its regulatory processes that control cell growth (17). Once activated, mTOR can stimulate protein synthesis through phosphorylation of the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and p70 ribosomal s6 kinase 1 (S6K1) to promote mRNA genesis and ribosomal protein translation (17). mTOR activation is known to cause attenuated levels of autophagy, which has numerous implications for growth of different cell types (27). Consequently, inhibitors of the mTOR complex such as rapamycin can be used to promote autophagy and are effective in treatment a variety of cancers.

DEPTOR and SCF$^{\beta\text{-TrCP}}$

Over the past several years a series of discoveries of protein regulators in the mTOR complex has led to significant increases in the understanding of the mechanisms of this process and provided potential candidate targets for disease treatment.

A relatively novel finding is that mTOR is regulated by the subunit binding protein known as DEPTOR, which inhibits mTOR expression upon reduction of mTOR activity and consequently decreases the rate of protein synthesis. While originally identified in cancer studies as a potent anti-cancer target, recent studies have only begun to explore DEPTOR's role in skeletal muscle regulation, and much remains to be discovered about

its role as a potentially key regulator of protein synthesis. DEPTOR is known to act as a negative regulator of mTOR activity by binding to mTOR and inhibiting its ability to affect downstream signals, leading to decrements in anabolic potential (15). By eliminating DEPTOR from cells via 'knockdown' (KD) procedures, it has been proven possible to increase cell size in some cell types (C2, MEF and HEK293) (15, 19). Additionally, DEPTOR can also promote cell autophagy by inhibiting the mTOR complex, which can serve as an intrinsic survival mechanism in cases of starvation and may hold additional implications as its role as regulator in cancer, diabetes, and muscle maintenance (10, 26, 27). Recent studies have identified the upstream regulator of DEPTOR as $SCF^{\beta\text{-}TrCP}$, an E3 ubiquitin ligase that acts as a negative regulator by targeting phosphorylated DEPTOR molecules for destruction by the proteosomal system (10, 25, 29). Increases in levels of intracellular $SCF^{\beta\text{-}TrCP}$ result in the activation of mTOR and subsequent increases in phosphorylation of downstream activation targets, as well as attenuation of autophagy (8, 10, 29). Consequently, $SCF^{\beta\text{-}TrCP}$ may also play a key role in muscle hypertrophy and maintenance, and as such should be considered in any research where DEPTOR expression is of interest.

The implications of DEPTOR's effects on protein synthesis and cell growth are of notable value, particularly concerning its potential application to the fields of diabetes, disuse, and obesity. Consequently, there is great interest in developing biotechniques to combat the muscle loss observed in these scenarios. It is possible that this muscle loss

may be induced by the same DEPTOR-induced mTOR inhibition pathway that leads to attenuated rates of protein synthesis. The future potential of DEPTOR inhibition to promote muscle growth in entire organisms is a subject of noteworthy scientific interest, and its application to medical and scientific fields could be highly significant.

CHAPTER II

METHODS

This research involves two phases: a literature review to evaluate the protocols using the Flexplate and other mechanical stretch stimulators to achieve various physiological outcomes for *in vitro* muscle tissue culture research and an analysis of different protocol outcomes for potential uses for evaluating DEPTOR signaling interactions.

Literature review

A literature review has been performed to compile a comprehensive understanding of the benefits, outcomes, and setbacks of various mechanical stretch protocols. Numerous approaches have been developed for replicating *in vivo* muscle contractions, including mechanical and electrical stimuli in both 2D and 3D constructs. Out of these protocols, the systems designed by The Flexcell International Corporation, particularly the Flexplate, have been well documented in muscle tissue culture research with a variety of methodologies and outcomes. This literature review uses resources published by numerous scholarly journals including the *Journal of Physiology*, *Journal of Biomechanics*, *American Journal of Physiology* and *The Biochemical Journal* to provide descriptions of the methodologies and technologies employed in numerous protocols as well as published outcomes and applications. This search provides comparisons between various tested approaches and the advantages and relevant published outcomes of each listed protocol.

Signaling analysis

To provide an opportunity for further future analysis of DEPTOR expression, an evaluation of various known pharmacological inhibitors of the mTOR signaling complex and their effects on downstream activation proteins and known outcomes has been given. This information is intended to facilitate an understanding of the molecular interactions governing protein synthesis regulation. By incorporating these inhibitors into desired exercise protocols, it may be possible to observe new outcomes that provide insight into the role of DEPTOR during exercise and its effects on muscle growth, signaling, and protein synthesis.

CHAPTER III

RESULTS

Literature review

This literature review involves providing a description of numerous selected protocols that have been used to achieve various experimental outcomes, including alterations in hypertrophy, mTOR signaling, and protein synthesis. While numerous approaches have been taken to simulate exercise *in vitro*, there exists a wide range of often-contradictory results that may be due to differences in exercise protocols and culture conditions. Due to these differences, an understanding of different options, approaches, and published outcomes of various protocols can be useful when designing an experiment to test one's own factor of interest. Here, comparisons between numerous aspects of experimental procedure including loading type, cell constructs, and exercise intensity are provided with the intent of enabling one to select the best approach for their desired outcome.

This literature review is divided into subsections detailing specific aspects of exercise protocol. Within each subsection are the supporting research for methods listed and the relevant physiological outcomes. Published research using inhibitory compounds in tandem with exercise protocol is also provided. Analysis of the listed approaches for potential maximization of observation of DEPTOR expression is provided, as well as the potential experimental benefits and impact of the addition of pharmacological inhibitory compounds.

2D vs. 3D cell constructs

Both 2D and 3D cell constructs are commercially available for mechanical stimulated exercise protocols. Because muscle cell development is influenced by local signaling and the surrounding extracellular matrix (ECM), the use of a 3D cell matrix hypothetically offers the potential to more effectively model cell maturation *in vivo*. Numerous studies using 3D gel matrices have observed muscle growth and differentiation with this model, including studies on human muscle tissue, cell lines, and MPCs (4, 20). While 3D cell constructs may be desirable for replicating the 3D cellular environment *in vivo,* numerous studies attempting to replicate the developmental processes and compare performance to *in vivo* tissue have been met with mixed results. A study by Boonen et al. observing genetic regulators of myogenesis and muscle maturation using cells grown and exercised on 2D and 3D cell constructs found no significant difference in maturation between the two groups (4). In a study by Powell et al. comparing human muscle *in vivo* vs. muscle *in vitro* cultured in 3D constructs and subjected to mechanical stimulation found that even after mechanical intervention, significant morphological differences were still present between the two muscle groups, even while using a 3D construct (2). However, studies by Atherton et al. and Hornenberger et al. using the 2D have provided promising results on muscle protein synthesis and mechanistic signaling processes, suggesting that 2D stretch, while perhaps not identical to an *in vivo* muscle cell environment, can provide relevant basic metabolic information for muscle growth and maintenance (2, 14).

While DEPTOR may be involved muscle maturation and development (although no studies to date have examined this case), the similarity in response of muscle growth to 2D and 3D cell constructs suggests that this type of environment may not be a key parameter to evaluate in initial studies looking to effects of DEPTOR expression. The success of experiments using 2D constructs in generating relevant post-exercise information on muscle protein synthesis and signaling transduction suggests that 2D cell constructs are a viable environment for observing DEPTOR's impact on muscle with exercise *in vitro.*

Uniaxial vs. multiaxial stretch

Different types of mechanical loading can alter the downstream reactions of striated muscle reacts. For example, heart muscle tissue undergoes differential changes in ventricular thickness in response to pressure loading versus volume loading (13). Additionally, in skeletal muscle, chronic longitudinal stretch produces a different cellular phenotype than overloading, which can be a factor to consider when optimal muscular performance is of interest (21). Due to muscle's ability to respond to a variety of mechanical inputs, employing different types of mechanical loading can be a means to invoke potentially different physiological responses. These opportunities are particularly valuable for *in vitro* studies considering the impracticality of isolating specific mechanical forces *in vivo,* where muscle is continually subjected to a wide variety of forces. Two common methods of force generation are uniaxial and multiaxial stretch systems, which use different types of mechanical deformation to stretch the

culture membranes. A study by Hornenberger et al. analyzed the effects of uniaxial and multiaxial stretch protocols on C2C12 downstream signaling responses (13). Two types of stretch protocols were employed: a 15% membrane cyclic stretch and cyclic stretch imparting 11% and 24% increases in surface area, both of which were produced at a frequency of 1 Hz in a pattern of 45 seconds exercise followed by 15 seconds rest for a total exercise time of 10 minutes (13). Multiaxial stretch alone produced significant increases in p70S6K phosphorylation 60 minutes post-exercise in both magnitudes of surface area deformation, suggesting that mutliaxial stretch may induce p70S6K phosphorylation through a novel mechanosensory pathway that may have implications toward muscle hypertrophy (13).

Considering the role of DEPTOR as an mTOR suppressor, the finding that multiaxial stretch induces p70S6K phosphorylation, a key downstream component of the mTOR pathway and positive regulator of muscle anabolism, may offer a promising scenario in which to analyze DEPTOR expression. Data from our own lab have shown an association between reduced DEPTOR expression with increases in p70S6K phosphorylation in studies of resistance exercise in rats (unpublished results). Considering that both scenarios present an increase in p70S6K expression, it may be possible that this mechanism is regulated by DEPTOR expression in multiaxial stretch and that this type of mechanical stimuli may impart similar reductions of DEPTOR expression.

Stretch intensity and duration

One of the primary advantages of a mechanical loading system is the precise amount of control one has over protocol intensity, bout, and duration. While exercise exertion can be regulated to an extent in living subject subjects, the ability to use mechanically regulated load parameters offers a much greater amount of control over exercise performance. It comes to little surprise that a wide variety of exercise parameters have been published with the intent of observing different outcomes in muscle cells. While earlier experiments in this field suggested limiting mechanical loading below 10% stretch to avoid muscle damage (23), the wide success of experiments using loads at or greater than that amount suggest that that concern may no longer be valid, possibly due to improvements in technology, changes in protocol, or a greater understanding of muscle function *in vitro*. Work by Atherton et al. using the L6 cell line demonstrated changes in mTOR activation and in sarcoplasmic and myofibrillar protein synthesis rates using a cyclic stretch protocol of 15% stretch at 1 Hz for 2, 15, or 30 minutes (2). In addition to samples taken from exercise bouts, samples were taken 30, 60, 90 and 120 minutes afterwards to analyze any post-exercise signaling effects. While no changes in sarcoplasmic protein synthesis from exercise were observed, myofibrillar protein synthesis decreased by 40% during exercise and gradually returned to basal rates post-exercise, which correlates with observations of muscle contractions *in vivo* (2). Analysis of post-exercise signaling mechanisms indicated surprisingly that positive regulators of anabolism, including mTOR, 4EBP1, ERK1/2, and p70S6K, exhibited elevated expression both during and after stretch and that the expression of suggested negative

regulators including AMPK and eEFK2 was conversely reduced (2). This seeming disconnect between protein synthesis and downstream signaling regulators may suggest that additional alternative roles or mechanisms of protein regulation are involved.

This protocol offers interesting potential for observation of DEPTOR expression. As an mTOR regulator, DEPTOR is associated with reductions in mTOR expression and data from our lab has found a negative correlation between DEPTOR expression and protein synthesis rates (unpublished results). It is possible that analysis of DEPTOR expression may provide an insight on the signaling transduction pathways involved during this exercise protocol and a greater understanding of the association between mTOR phosphorylation and protein synthesis rates.

Work by Sasai et al. using cultured chick embryo myotubes subjugated to 10% cyclical stretch at 1 Hz for 72 hours or 5-60 minutes also demonstrated changes in mTOR signaling in response to mechanical loading as well as the addition of known inhibitors of the mTOR pathway (22). Subjection of muscle cells to cyclic stretch protocols for 72 hours resulted in myotube hypertrophy, and 5-60 minutes of mechanical stimulation resulted in significant increases in downstream Akt phosphorylation (22). To investigate the activation of the PI3K/Akt/TOR pathway, muscle samples were exposed to a combination of exercise and pharmacological inhibitory compounds, including wortmanin, an inhibitor of PI3K, rapamycin, an mTOR inhibitor, and U0126, an inhibitor of MEK/ERK. Addition of rapamycin produced significantly greater decreases

in mTOR expression in stretched versus unstretched cultures, suggesting that mTOR may be activated during mechanical stretch, which would be supportive of the findings proposed by Atherton et al (2). Similarly, wortmannin caused a significant reduction in myotube diameter in stretched cultures only, suggesting that PI3K may also be involved in exercise-stimulated muscle hypertrophy (22). Surprisingly, U0126 caused increases in both stretch and unstretched myotube diameters, which suggests that MEK/ERK may not be essential for muscle hypertrophy and may be a potential downregulator of myotube diameter after differentiation (22).

The use of pharmacological inhibitors with and without stimulated exercise offers a great potential to observe the signaling interactions and role of DEPTOR in muscle anabolism and response to exercise. As Sasai et al. used pharmacological inhibitors of mTOR to observe the effects of exercise on its expression, using a similar protocol targeted towards DEPTOR expression may offer an opportunity to analyze its signaling interactions. While no known specific pharmacological inhibitors of DEPTOR itself have been published, KD studies in conditions of sepsis have shown that altering DEPTOR expression can impart a positive physiological effect on muscle mass (15). However, it has been shown possible to target DEPTOR expression through the inhibition of its upstream inhibitor, $SCF^{\beta\text{-}TrCP}$ MLN4924, a pharmacological inhibitor of $SCF^{\beta\text{-}TrCP}$ has been recently identified to cause downstream reductions in DEPTOR expression in cancer cells (28). While this drug's effects have yet to be tested in muscle,

the similarities in mTOR regulation between these two cell types suggest that MLN4924 may have similar effects in muscle, and in that case, would be an ideal means for targeting DEPTOR expression through its upstream signaling. Rapamycin, an inhibitor of mTOR, is also a potentially promising pharmacological candidate for observing mTOR and DEPTOR interaction, considering how mTOR actively phosphorylates DEPTOR in order to target it for destruction by $SCF^{\beta\text{-}TrCP}$ and the proteosomal system (8, 10).

CHAPTER IV

CONCLUSIONS

This research had the goal of analyzing several approaches to mechanical exercise stimulation in cell culture and targeting their potential application to the study of DEPTOR expression and regulation. Due to the wide variety of options, variations, and technologies available for mechanical exercise stimulation and protocol, designing an approach to maximize the potential for observing an outcome of interest can be a complex process. Here, several factors of a mechanically simulated exercise protocol were compared and assessed for potential use in future studies targeting DEPTOR expression.

In the first comparison, experiments using 2D and 3D cell constructs were assessed and evaluated for physiological relevance. In one study, no significant developmental changes were observed between cells grown in 2D vs. 3D constructs, suggesting that this type of environment may not be a critical determining factor of muscle cell maturation. Furthermore, the relevant physiological outcomes derived from studies using 2D cell constructs suggests that a 2D environment is sufficient for generating data on basic anabolic processes including protein synthesis regulation and signal transduction.

A second comparison between uniaxial and multiaxial stretch was performed to evaluate the potential different outcomes due to differences in the type of mechanical load. Work

by Hornenberger et al. indicated that muscle cells respond differently to changes in mechanical load due to a mechano-sensory mechanism. Findings from this study showed an increase in downstream p70S6K phosphorylation in response to multiaxial stretch, suggesting that multiaxial stretch may induce an alternative pathway for muscle hypertrophy. Considering DEPTOR's effect on p70S6K expression, the findings generated by Horenberger et al. may provide a basis for a scenario in which to test DEPTOR expression as a means to provide insight on the signal transduction induced this type of mechanosensory pathway.

Finally, comparisons between stretch intensity and duration were performed to provide insight onto the impact of different stretch protocols on mTOR activation and protein synthesis. In accordance with observations of muscle protein synthesis *in vivo*, cyclic stretch exercise protocols performed by Atherton et al. caused a reduction in myofibrillar protein synthesis rates during exercise followed by a gradual increase and return to basal level following exercise. Signaling analysis also revealed that this exercise protocol caused an increase in anabolic signaling during and after exercise, which suggests additional roles for this pathway during scenarios of exercise. Considering the role of DEPTOR on mTOR expression and protein anabolism, repetition of a study using these parameters may provide new insight on the role of DEPTOR in protein synthesis and may possibly explain the unexpected signaling events encountered during the study.

Many opportunities exist for evaluating and manipulating DEPTOR expression in response to exercise, and pharmacological inhibition of mTOR and $SCF^{\beta\text{-}TrCP}$ are two promising means of altering DEPTOR expression. By combining the use of these pharmacological inhibitors with simulated exercise, it may be possible to gain greater insight into the role of DEPTOR on protein synthesis, signaling regulation, and ultimately muscle function and maintenance.

These analyses demonstrate how changes in mechanical exercise protocol can affect muscle's response to various stimuli and provide an understanding of what approaches are available to achieve various outcomes for studies on DEPTOR analysis. Future research in this field should focus on designing a protocol specific for DEPTOR expression and regulation of its upstream inhibitors. Use of pharmacological inhibitors in combination with exercise protocols may provide new understanding of the signaling pathways involved in exercise and the role of DEPTOR in governing muscle anabolism. A greater understanding on DEPTOR's role in protein synthesis and anabolism during exercise could significantly expand our knowledge of muscle function and regulation. Considering DEPTOR's additional role in cancer development and progression, advances made in the understanding of this molecule's function and regulation could be a key step in developing treatment a variety of metabolic diseases.

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Figures:

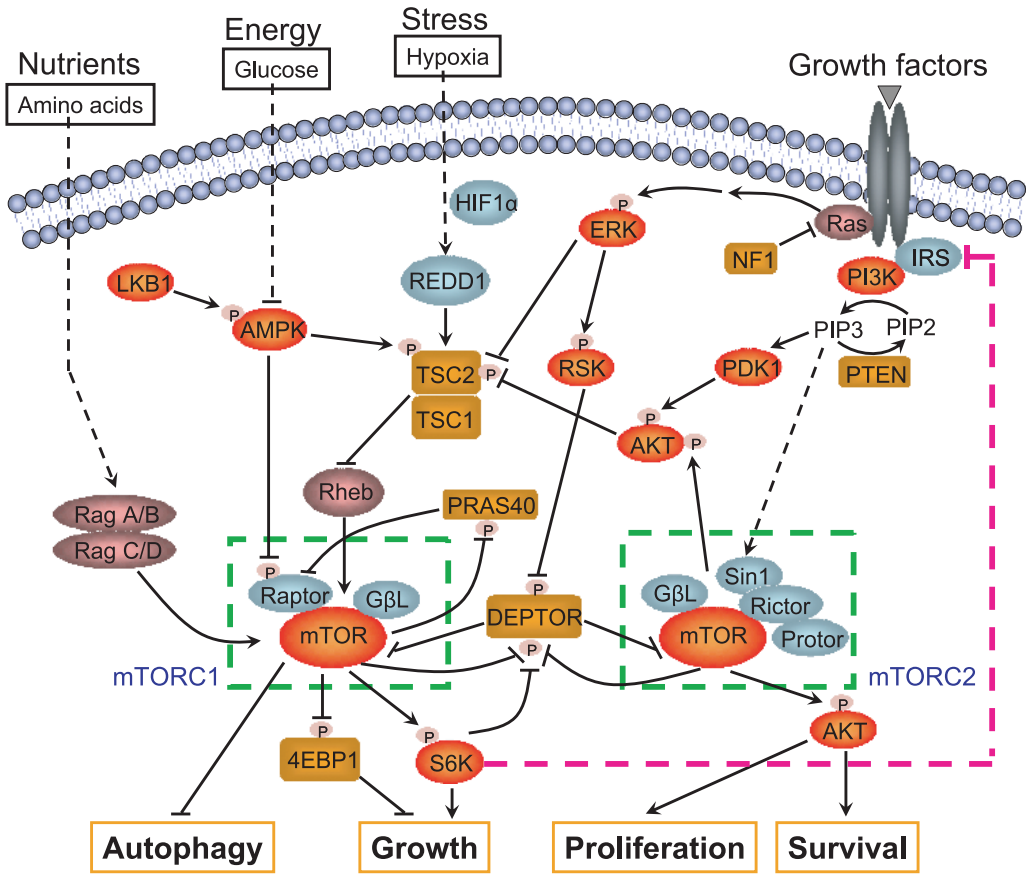

Figure 1: Illustration of the mTOR signaling transduction pathway. DEPTOR is a central regulator of both mTORC1 and mTORC2. (Figure from Zhao et al., 2012).

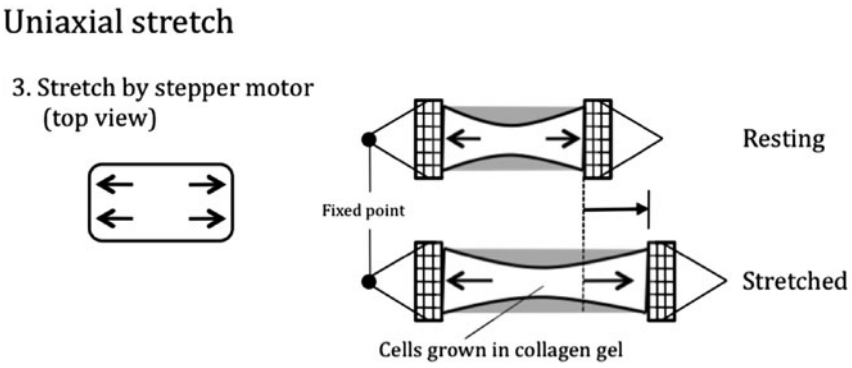

Figure 2: A schematic representation of a uniaxial flex system. Force is generated in a linear plane to mimic muscle contractions on cells grown on a matrix. (Figure from Passey et al., 2011).